

Amendments

This listing of claims will replace all prior versions, and listings of claims in the application.

1-112 (Canceled).

113. (New) A nucleic acid molecule comprising, in order:

- (a) an origin of replication,
- (b) a first positive selection marker,
- (c) a first promoter,
- (d) a first site-specific recombination site,
- (e) a coding sequence,
- (f) a second positive selection marker,
- (g) a second site specific recombination site, and
- (h) a second promoter;

wherein the first promoter is operably linked to the coding sequence, wherein the second promoter is operably linked to the second positive selection marker.

114. (New) The nucleic acid molecule of claim 113, wherein the first and second site-specific recombination sites are selected from the group consisting of a *lox* site, a lambdoid *att* site, and mutants thereof.

115. (New) The nucleic acid of claim 113, wherein the first and second site-specific recombination sites are *lox* sites.

116. (New) The nucleic acid molecule of claim 115, wherein the *lox* sites are a *loxP* sites.

117. (New): The nucleic acid molecule of claim 113, wherein said nucleic acid molecule further comprises at least one multiple cloning site.

118. (New) The nucleic acid molecule of claim 113, wherein said nucleic acid molecule is a vector.

119. (New) The nucleic acid molecule of claim 118, wherein said vector is an expression vector.

120. (New) The nucleic acid molecule of claim 113, wherein the first and second positive selection markers are antibiotic resistance genes.

121. (New) The nucleic acid molecule of claim 120, wherein the antibiotic resistance genes are selected from the group consisting of a chloramphenicol resistance gene, an ampicillin resistance gene, a methicillin resistance gene, a tetracycline resistance gene and a kanamycin resistance gene.

122. (New) The nucleic acid molecule of claim 120, wherein the first and second positive selection markers are different antibiotic resistance genes.

123. (New) The nucleic acid molecule of claim 120, wherein the second antibiotic resistance gene is a chloramphenicol resistance gene.

124. (New) A host cell comprising the nucleic acid molecule of claim 113.

125. (New) A nucleic acid molecule comprising, in order:

- (a) a first site-specific recombination site,
- (b) a negative selection marker,
- (c) a positive selection marker,
- (d) a second site-specific recombination site,
- (e) a second positive selection marker;

wherein the first positive selection marker and the second positive marker are different.

126. (New) The nucleic acid molecule of claim 125, wherein the first and second site-specific recombination sites are selected from the group consisting of a *lox* site, a lambdoid *att* site, and mutants thereof.

127. (New) The nucleic acid molecule of claim 125, wherein the first and second site-specific recombination sites are *lox* sites.

128. (New) The nucleic acid molecule of claim 127, wherein the *lox* sites are a *loxP* sites.

129. (New) The nucleic acid molecule of claim 125, wherein the negative selection marker is a restriction endonuclease site.

130. (New) The nucleic acid molecule of claim 129, wherein the restriction endonuclease site is *DpnI*.

131. (New): The nucleic acid molecule of claim 125, wherein said nucleic acid molecule further comprises at least one multiple cloning site.

132. (New) The nucleic acid molecule of claim 125, wherein said nucleic acid molecule is a vector.

133. (New) The nucleic acid molecule of claim 132, wherein said vector is an expression vector.

134. (New) The nucleic acid molecule of claim 125, wherein the first and second positive selection markers are antibiotic resistance genes.

135. (New) The nucleic acid molecule of claim 132, wherein the antibiotic resistance genes are selected from the group consisting of a chloramphenicol resistance gene, an

ampicillin resistance gene, a methicillin resistance gene, a tetracycline resistance gene and a kanamycin resistance gene.

136. (New) The nucleic acid molecule of claim 120, wherein the second antibiotic resistance gene is a chloramphenicol resistance gene.

137. (New) A host cell comprising the nucleic acid molecule of claim 125.

138. (New) The host cell of claim 137, wherein the host cell is an *Escherichia coli* cell.

139. (New) A nucleic acid molecule comprising, in order:

- (a) an origin of replication,
- (b) a first positive selection marker,
- (c) a first site-specific recombination site,
- (d) a second positive selection marker,
- (e) a third positive selection marker, and
- (f) a second site-specific recombination site,

wherein the first positive selection marker, the second positive marker and the third positive selection marker are different from each other.

140. (New) The nucleic acid molecule of claim 139, wherein the first and second site-specific recombination sites are selected from the group consisting of a *lox* site, a lambdoid *att* site, and mutants thereof.

141. (New) The nucleic acid of claim 139, wherein the first and second site-specific recombination sites are *lox* sites.

142. (New) The nucleic acid molecule of claim 141, wherein the *lox* sites are a *loxP* sites.

143. (New): The nucleic acid molecule of claim 139, wherein said nucleic acid molecule further comprises at least one multiple cloning site.

144. (New) The nucleic acid molecule of claim 139, wherein the nucleic acid molecule is a vector.

145. (New) The nucleic acid molecule of claim 144, wherein the vector is an expression vector.

146. (New) The nucleic acid molecule of claim 139, wherein the first, second and third positive selection markers are antibiotic resistance genes.

147. (New) The nucleic acid molecule of claim 146, wherein the antibiotic resistance genes are selected from the group consisting of a chloramphenicol resistance gene, an ampicillin resistance gene, a methicillin resistance gene, a tetracycline resistance gene and a kanamycin resistance gene.

148. (New) A host cell comprising the nucleic acid molecule of claim 137.

149. (New) The host cell of claim 148, wherein the host cell is an *Escherichia coli* cell.